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Pathological study of experimentally induced tick bitten (*Argas persicus*) in poultry skin

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Abstract

The fowl blood sucking tick "*Argas persicus*" is of great medical and veterinary importance in tropical and subtropical regions because of its role as the vector of certain parasitic bacterial and viral pathogens. In this study, the pathological changes of its bite on the poultry skin have been investigated. Twenty two (12 infested with adults and 10 infested with nymphs) *Ross broilers* (308) were infested with the tick on the skin of hock joints. Other side healthy legs were used as control. Samples were collected after 6, 24, 48 and 96 hours and 1 or 2 weeks. The skin samples were fixed at 10% buffered formalin and histological sections were prepared using routine Haematoxylin and Eosin staining method. The results showed subcutaneous oedema, massive infiltration of lymphocytes, extensive hemorrhage, feather follicle oedema. Necrosis and epidermal hyperplasia are the prominent lesions in this study. It is concluded that the chicken infestation with *Argas persicus* caused cutaneous and subcutaneous lesions. Therefore, it can be considered as a cause of economic loss.

Keywords: *Argas persicus*, Poultry, Pathologic changes, tick biting, cutaneous lesions

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Introduction

The ectoparasites of poultry like ticks play an important role in the transmission of a certain pathogen which causes heavy economic losses to the poultry industry. They cause heavy morbidity by sucking blood and causing irritation to the birds which adversely affects the economical production of poultry (Phulan *et al*., 1984). It has a worldwide distribution in warm climates and also it is prevalent in different parts of Iran. Apart from causing anemia, anorexia, weight loss and decreased egg production, Argas persicus is the main vector of Borreliaanserina (the causal agent of avian spirochetosis) and Aegyptianellapullorum (Adamu *et al.,* 2014). It is also capable of transmitting Mycobacterium avium, Pasteurella avicida/multocida, West Nile virus, Salmonella gallinarum/pullorum, Mycoplasma gallisepticum/meleagridis to poultry (Stefanov *et al.*, 1975; Soliman *et al.*, 1988). Tick paralysis in chickens, a flaccid motor paralysis, may result from attacks by the nymph stage of Argas persicus ((Rosenstein, 1976). In addition to chickens and other domestic fowls, it also feeds on humans (Trager, 1940). The optimum temperature for the development of Argas persicus is 22-38°C (Petrov *et al.*, 1975).This development cycle of Argas persicus is completed in 41-133 days (Petrov *et al.*, 1975; Srivastava *et al.*, 1981) aand 13-38 days at room temperature and humidity (Abbas *et al.*, 2004).

The insertion of the tick hypostome into the host skin causes damage to the epidermis and rupture of blood vessels. However, tick saliva contains compounds that counteract host hemostatic, inflammatory, and immune responses and enable ticks to feed for days to weeks at one site (Ribeiro *et al.*,1990; Andrade *et al.*, 2005; Steen *et al.*, 2006). This study was done to evaluate pathological changes of poultry skin caused by nymph and mature Argaspersicus biting in various periods of times.

Material and methods

Sample collection and identification

The *Argaspersicus* ticks are active during the night. They spend the daytime hidden in cracks and crevices of the walls of chicken houses or wooden materials such as windows or doors of poultry-roosting areas. Therefore, these places were examined for the presence of ticks in some villages of Urmia city, west Azerbaijan of Iran. Special attention was paid to the feces of the ticks which were in the form of black and red grains like blood clots in the tick habitats. At each infested site, several specimens at different developmental stages were found, and then they were transferred to the department of parasitology, faculty of veterinary medicine of the Urmia University where their species and sex have been determined based on using morphological characteristics (Wall *et al.*, 1997). The ticks were kept at room temperature with 80% relative humidity inside desiccators. Some adult engorged female ticks were kept in separate desiccators at room temperature (22-25°c) with 80% RH for egg production. The eggs laid during 2 weeks were collected and kept in desiccators to obtain nymph.

Infestation of chicks with the Argaspersicus

Twenty –two 4 weeks old healthy *broilers* (*Ross, 308*) were prepared from *Urmia* poultry farms. They were kept in metal cages and allowed to acclimatize for 7 days.

The macroscopic evaluations confirmed that the chicks were ectoparasite free. They were divided into the two groups.

One group (n=12) was infested with 10 adult tick while the other group (n=12) was infested with 30 nymph.

The chicks were infested with the tick on the skin of right hock joints. Left leg hock joints skin were used as control.

For prevention of tick migration, the region was covered with a bandage.

In the group infested with adult tick

samples were collected in 6, 24, 48 and 96 hours and 1 and 2 weeks after euthanasia (Two samples from this group at each time period). Sampling was carried out according to previous studies (Hobbenaghi *et al.*, 2012; Gholizadehet *et al.*, 2015). Samples were taken from sites that were mild papula in biting location and red pointed.

And in the group infested with nymph, samples were collected after 6, 24, 48, 96 hours and 1 and 2 weeks after euthanasia (Two samples from this group at each time period).

The skin was immediately immersed in fixative (10% buffered formalin). After fixation, it was embedded in paraffin, sectioned in 5µm thin slices, stained with Hematoxylin and Eosin (H&E) and studied by a routine light microscope.

Results

In infestation with adult tick: at 6, 24 hours, subcutaneous edema and hemorrhage in feather follicles, at 48 hours, subcutaneous edema along with the centers of accumulation of Heterophil and necrosis were observed. At 96 hours, subcutaneous oedema, hemorrhage and lymphocyticin filtration were seen. Skin folding initiated in 48 hours (Fig. 2).

At 1-2 weeks, hemorrhage and focal lymphocytic infiltration in sub cutis, hydropic degeneration and some degrees of necrosis were seen in feather follicles (Fig.1). In some areas of epidermal hyperplasia it was seen that in this area the number of cell layers was more than that of other area (Fig. 4).

In infestation with nymph: at 6 hours, subcutaneous edema and extensive hemorrhage, at 24 hours, widespread edema, severe hemorrhage and Heterophil infiltration, at 48 hours, perivascular extensive hemorrhage and necrosis of feather follicles (Fig.3), and at 96 hours subcutaneous edema, extensive hemorrhage and foci of lymphocyte infiltration and necrosis were seen (Fig.1).

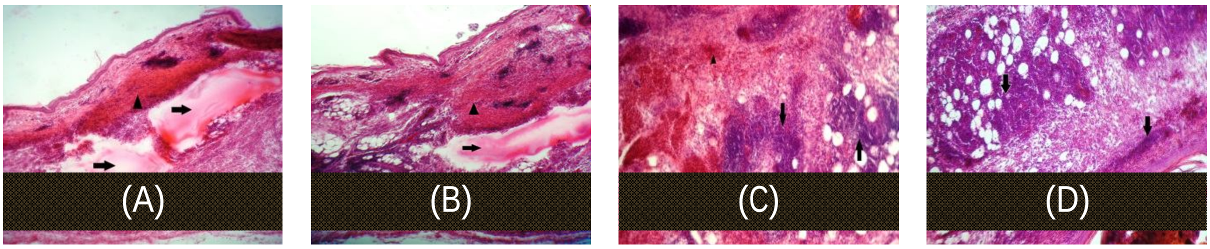


Figure 1. Hemorrhage and lymphocyte infiltration in the dermis, H&E staining (×100); (A) Hemorrhage (arrow heads) extensive edema (arrows) in the dermis due to the adult tick bites in 1 week. (B) Hemorrhage (arrow heads) and extensive edema (arrows) in the dermis due to the nymph in 96 hours (C) Massive lymphocytic infiltration (arrows) and hemorrhage (arrow heads) in the dermis due to the nymph in 1 week. (D) Massive lymphocytic infiltration (arrows) in the dermis due to the nymph (White arrow) in 2 weeks.

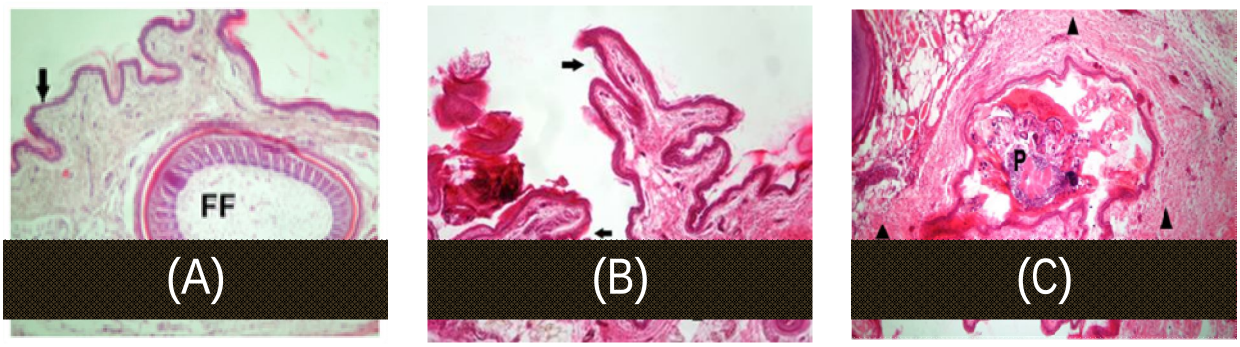


Figure 2. H&E staining of the skin. (A) Normal skin layers (arrow) and a feather follicle (FF) in healthy leg as a control (×100). (B) Epidermal folding (arrows) and edema (arrows head) in sub cutis due to the adult ticks infestation (×100). (C)

Epidermal folding (arrows) and increase the subcutaneous connective tissue (arrows head) due to the nymph (P) irritation



Figure3. Stages of feather follicles degeneration and Necrosis due to the nymph infestation (H&E staining). (A) Hemorrhage in feather follicle (arrow) and its surroundings (arrows head) (×100). (B)Degeneration of Feather follicle epidermis layers in 1 week (arrow).arrow head show the normal Feather follicle layers (×100). (C) Necrosis of Feather follicle epidermis layers in 2 weeks (arrow) (×100).

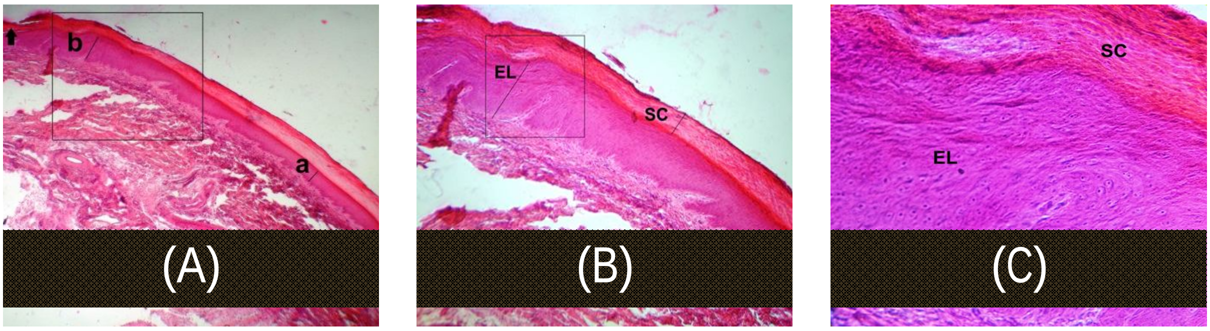


Figure4. Hyperplasia of the epidermis due to the adult tick bites in 2 weeks. The number of epidermis layers (EL) to stratumcorneum (SC) has increased in some areas (b) H&E staining; A, ×40, B, ×100 and C, ×400.

At 1 week, subcutaneous edema and foci of necrosis and extensive multifocal hemorrhages (Fig.1) also Epidermal folding with increase the subcutaneous connective tissue were seen (Fig.2), and at 2 weeks subcutaneous edema, necrosis of feather follicles (Fig.3) and feather loss was prominent.

A comparison the quality of lesions caused by mature tick and nymphin over time is shown in the Table1.

Discussion

According to the results in table 1 and the figure1, vascular lesions including edema and hemorrhage were prominent pathological changes of *Argaspersicus* biting. Based on Bowman and Sauer (Bowman *et al*., 2004), following laceration of blood vessels by ticking mouth parts, Arachidonic acid is released by the activated platelets and it is converted into Thromboxane A2 and it causes platelet aggregation-degranulation and vasoconstriction. To antagonize vasoconstrictors produced by the host on the site of tissue injury, vasodilators are secreted by ticks to the feeding pool (Ribeiro *et al*., 1998; Bowman *et al*., 2004).In this study, the presence of subcutaneous edema in several cases indicates an increasing vascular permeability due to vascular damage by the blood sucking activity of the tick and its secreted vasodilators in the feeding pool.

Hemorrhage was also a most prominent finding of tick biting by both adult and nymph. Several specific direct thrombin inhibitors have been characterized in salivary of ticks (Hoffmann *et al*., 1991; Zhu *et al*., 1997; Nienaber *et al*., 1999; Horn*et al*., 2000; Iwanaga *et al*., 2003; Motoyashiki *et al*., 2003).The saliva of the same tick species contains simultaneously more antihemostatic system (Arocha-Pinango *et al*., 1999).

Table 1. Type and quality of lesions and severities caused by the nymph and adult of *Argas Persicus* on poultry skin.

Type of lesions	stages of tick	Time interval and severity of lesions					
		6 hours	**24 hours**	**48 hours**	**96 hours**	**1 week**	2 weeks
Edema	adult	−	++	++++	++++	+++++	+++++
	nymph	−	++	+++	++++	++++	++++
Lymphocyte infiltration	adult	−	−	++	++++	++	+++
	nymph	−	−	−	−	+++++	+++++
Hemorrhage	adult	+	++	+++	++++	++++	++++
	nymph	−	++	+++	+++++	+++++	+++++
Feather follicle edema	adult	−	+	+	++	+++	++++
	nymph	−	−	−	−	++++	++++
follicle degeneration & necrosis	adult	−	−	−	++	++	+++
	nymph	−	++	++	++	+++	+++++
skin folding & hyperplasia	adult	−	+	++	+++	+++++	+++++
	nymph	−	+	++	+++	++++	++++
Heterophil infiltration	adult	−	−	+++	+++	++	++
	nymph	−	−	+++	+++	++	++

Lymphocyte infiltration in the samples indicates that the released materials into the subcutaneous induce an immune response and in the long time, gradually lymphocyte infiltration is more common. However, since it occurs in infestation with *Argas reflexus*, eosinophil infiltration was not observed (Gholizadeh *et al*., 2015). However, based on some research reports, ticks produce substances that inhibit the pro-inflammatory functions of most infiltrating cells at the attachment site, e.g., neutrophils (Ribeiro *et al*., 2003), NK cells (Kubes *et al*., 1994)(), Macrophages, T cells (Ramachandra et al., 1992; Bergman *et al*., 2000), and Dendritic cells (Cavassani *et al*., 2005).

In this study heterophil infiltration was seen after 48 hours. These finding showed that salivary materials of *Argas persicus* can reduce the immune response and prevent heterophil infiltration in the early stages of the infection. In addition, attenuation of immune mechanisms may also enhance the transmission of tick borne pathogens (Schoeler *et al*., 2001; Wikel *et al*., 2001)( ) and therefore lead to the aggregation of heterophils.

Ticks can induce injury in dermal vessels while biting and they can cause vascular hemorrhage and therefore disturbances of subcutaneous blood supply. Circulatory disturbances can explain the pathological changes in feather follicles such as hydropic degeneration and necrosis. This is general histopathological findings in which cells absorb much water and this occurs in response to the loss of the cells homeostasis secondary to mechanical, hypoxic, toxic, free radical, viral, bacterial, and immune-mediated injuries (James F. Zachary, 2012). Necrosis of feather follicle and feather follicle loss that has already been seen in our previous studies and infestation with red mite of poultry, *Dremanyssus gallinae*, was observed in this study too. *(*Hobbenagh*i et al., 2012).()*

These findings showed that the tick biting caused cutaneous damage consisting of edema, cellular infiltrations, and extensive hemorrhage. These lesions predispose the animals to lose body weight, decrease food uptake and economic loss. Moreover, the bad appearance of the carcass reduces marketability and this is a point that must be carefully considered in the poultry industry.

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بررسی تجربی اثرات پاتولوژیک گزش کنه آرگاس پرسیکوس بر روی پوست ماکیان

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چکیده

کنه خونخوار ماکیان(*آرگاس پرسیکوس*) اهمیت بسزایی در پزشکی ودامپزشکی هم در نواحی گرمسیری وهم در نواحی تحت استوایی به جهت نقش آن در انتقال عوامل پاتوژن خاص میکروبی و ویروسی دارد.در این مطالعه تغییرات پاتولوژیک حاصل از گزش آن در پوست ماکیان مورد بررسی قرار گرفته است.22 جوجه گوشتی نژاد راس (308) ، (12 مورد برای آلودگی انگلی با فرم بالغ کنه و10 مورد هم برای آلودگی با لارو کنه) در پوست پا در ناحیه اطراف مفصل خرگوشی در معرض آلودگی با کنه قرار داده شدند.پا های سمت مقابل بعنوان کنترل مورد استفاده قرار گرفتند. نمونه گیری از پوست ناحیه بعد از 6 ،24 ، 48 ساعت ویک و دو هفته انجام شد.نمونه ها در فرمالین 10% بافر قرار داده شد واز نمونه ها مقاطع میکروسکوپی با رنگ آمیزی هماتوکسیلن-ائوزین تهیه گردید.نتایج نشان دهنده ایجاد ادم زیرجلدی ،نفوذ شدید لنفوسیتی وخونریزی گسترده و ادم در فولیکول پر و نکروز در این ناحیه وهمچنین هیپرپلازی اپیدرم می باشد.

این نکته استنتاج می شود که آلودگی ماکیان با کنه *آرگاس پرسیکوس* باعث ضایعات جدی در پوست و زیر پوست شده و می تواند باعث خسارتهای اقتصادی فراوانی در صنعت مرغداری شود.

واژگان کلیدی: آرگاس پرسیکوس، تغییرات پاتولوژیک، گزش کنه، ضایعات جلدی